

Attraction and Feeding Responses of Melon Flies and Oriental Fruit Flies (Diptera: Tephritidae) to Various Protein Baits with and without Toxicants¹

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Abstract: Studies were conducted to determine attraction and feeding propensity of oriental fruit fly, *Bactrocera dorsalis* (Hendel), and melon fly, *Bactrocera cucurbitae* (Coquillett) to different protein bait mixtures with and without the insecticides spinosad and malathion. The type of protein bait (Provesta®621autolyzed yeast extract, Mazoferm®E802, GF-120®Fruit Fly Bait, or Nu-Lure®Insect Bait) had a major influence on *B. dorsalis* and *B. cucurbitae* attraction and feeding, which was strongest to fresh Provesta, GF-120, and Mazoferm. There was no significant response to bait aged for 4 d. In feeding propensity studies, highest response was observed for Mazoferm. On the basis of attraction and feeding responses Provesta (attraction) and Mazoferm (feeding) outperformed the standard Nu-Lure. A mixture of Provesta and malathion was significantly less attractive to *B. dorsalis* and *B. cucurbitae*, compared to a mixture of Provesta and spinosad. Our studies suggest that protein-starved *B. dorsalis* flies were much more likely to feed on protein compared to protein-fed flies. Spinosad has low contact toxicity, and, when mixed with protein baits, offers a reduced risk alternative for control of *B. dorsalis* and *B. cucurbitae*, without many of the nontarget effects of broad spectrum contact poisons such as malathion.

Key words: Spinosad bait, malathion bait, *Bactrocera dorsalis*, *Bactrocera cucurbitae*, control.

Introduction

Bactrocera is a tephritid fly genus of at least 440 species distributed primarily in tropical Asia, the south Pacific, and Australia (White and Elson-Harris 1992). *Bactrocera cucurbitae* (Coquillett), melon fly, is a serious agricultural pest of cucurbits. It has been recorded from over 125 plant species (Weems 1964) and is found in India, Myanmar, Malaysia, Thailand, the Philippines, southern China, Taiwan, east Africa, Guam, the Commonwealth of the Northern Mariana Islands, Papua New Guinea, Solomon Islands, Nauru, and the Hawaiian Islands (Nishida 1953, White and Elson-Harris 1992). In 1895 it was discovered in Hawaii (Back and Pemberton 1917), where it causes serious economic damage to cultivated species of Cucurbitaceae (e.g. cucumber, *Cucumis sativus* L.; watermelon, *Citrullus lanatus* (Thunb.) Matsum. & Nakai; cantaloupe, *Cucumis melo* L.; pumpkin, *Cucurbita maxima* Duchesne ex Lam.; cultivated bitter melon (balsam pear), *Momordica charantia* L.; and zucchini, *Cucurbita pepo* L.) (White and Elson-Harris 1992). When populations are high and cucurbits scarce, *B. cucurbitae* also attack, but less frequently, other species of vegetables and fruits, such as papaya (*Carica papaya* L.).

Bactrocera dorsalis (Hendel), oriental fruit fly, is found throughout Asia, including Bhutan, southern China, India and Thailand, and has been recorded from over 173 host species (White and Elson-Harris 1992). It was introduced into Hawaii in 1945 and is now the most

abundant and widely distributed fruit fly in the islands. Studies have indicated that 95% of the population develops in common guava, *Psidium guajava* L., and strawberry guava, *P. cattleianum* Sabine, and that population cycles are determined primarily by wild guava fruiting (Newell and Haramoto 1968; Vargas et al. 1983). Commercial and backyard fruits are severely damaged by *B. dorsalis* increases in nearby guava patches. Because of the abundance of common and strawberry guava throughout Hawaii, *B. dorsalis* has played a direct role in inhibiting the development of a profitable, and diversified tropical fruit industry (Vargas, et al. 2000).

Traditionally, fruit flies have been controlled in agricultural areas of Hawaii using protein bait sprays. Female flies need protein for full ovarian development and egg production, thus they readily feed on a protein source containing a toxicant. The bait spray strategy dramatically reduces the amount of pesticide needed for fruit fly control and has been used successfully in eradication campaigns (Steiner et. al. 1961, Roessler 1989).

Since the late 1950s the most common toxicant used in fruit fly bait spray formulations has been the organophosphate insecticide malathion (Roessler 1989). Nu-Lure has been the most popular protein bait mixed with malathion for fruit fly control (Prokopy et al. 1992). However, organophosphate insecticides have been implicated in negative effects on natural enemies and human health (Carson 1962). Recently, new bait spray formulations containing reduced risk insecticides, such as spinosad or phloxine B, have been tested for use in the U.S. (McQuate et al. 1999, Peck and McQuate 2000).

Spinosad, a toxin derived from the soil dwelling actinomycete bacterium *Saccharopolyspora spinosa* Mertz and Yao, has low mammalian toxicity, and reduced environmental impact on natural enemies (Stark et al. 2004). A hydrolyzed protein bait with spinosad that attracted, induced feeding, and killed fruit flies was initially developed by Moreno and Mangan (1995). The first bait contained liquid Mazoferm E802TM (an enzymatically hydrolyzed protein from corn processing (Corn Products, Argo IL)) as the protein component. This bait was shown to have little impact against a series of beneficial hymenopteran parasitoids (Dowell 1997) and reduced impact against honeybees (Dominguez et al. 2003). Vargas et al. (2002) demonstrated that the fruit fly parasitoid, *Fopius arisanus* (Sonan), did not readily feed on a protein bait droplets with spinosad. Further tests are underway examining the impact of GF-120 on nontarget insects in the wild. The Mazoferm bait was field tested against Mediterranean fruit fly, *Ceratitidis capitata* (Wiedemann), in Hawaii (Peck and McQuate 2000, Vargas et al. 2001). A second bait composed of Solulyls, a dried and more purified product processed from Mazoferm, was developed and successfully tested with a series of toxicants. This bait, Solbait, contained the hydrolyzed protein, Solulyls, as the protein attractant compound and has since been produced as GF-120 Fruit Fly Bait (Dow AgroSciences, Indianapolis, IN)(DowElanco 1994).

In 1999, USDA-Agricultural Research Service funded the Hawaii Fruit Fly Area-Wide Pest Management program. One of the major objectives of the program was to suppress fruit flies below economic thresholds, while reducing the use of organophosphate insecticides on farms (Vargas et al. 2003). The program includes developing and integrating biologically based pest control technology into a comprehensive management package that will be economically viable, environmentally friendly and sustainable. Major components include: 1) field sanitation, 2) GF-120 Fruit Fly Bait (Vargas et al. 2003), 3) male annihilation (Vargas et al. 2000), and if needed, 4) augmentative parasitoid releases, and 5) sterile insect releases. Thus far GF-120 Fruit Fly Bait has been very useful for control of *B. cucurbitae* (Prokopy et al. 2003; 2004); however, it has not been extensively tested against *B. dorsalis*. These were the first tests of spinosad baits against *B. dorsalis* and *B. cucurbitae*. Here we report results of behavioral studies of both *B. dorsalis* and *B. cucurbitae* to Nu-Lure and alternative baits, both with and without spinosad and malathion. We divided our

study into local attraction experiments conducted in field cages and direct feeding behavior conducted with individual flies in the laboratory.

Materials and Methods

Insects. *Bactrocera cucurbitae* and *B. dorsalis* pupae were obtained from a colony maintained for approximately 360 and 137 generations, respectively, at the USDA-ARS, United States Pacific Basin Agricultural Research Center (USPBARC) in Honolulu, HI (Vargas 1989). Pupae were shipped to the USPBARC in Hilo, HI, and flies allowed to emerge inside plastic boxes (32 x 58 x 49 cm). All tests took place at the University of Hawaii research station at Kainaliu, HI.

Attraction Experiment 1. Tests with *B. dorsalis* were conducted on March 15 and 16, 2000. Tests with *B. cucurbitae* were done on March 21 and 23, 2000. Flies of both species were tested in two different physiological states. Protein-fed flies were fed a diet (3:1 by volume) of sugar and ICN enzymatic yeast hydrolysate (United States Biochemical, Cleveland, Ohio), protein-deprived flies were fed sugar only. Flies in each group were provided water *ad libitum* and held inside a room at $22 \pm 2^\circ \text{C}$, 40-90% r. h., and a 12:12 (L:D) h photoperiod until eclosion. Flies were tested when they were 10–12 d old.

Replicate trials were carried out inside lumite screen field cages (3 m tall x 3 m diameter) positioned inside a shade house (Prokopy and Vargas 1996, Vargas et al. 2002). Each of four large field cages contained five potted guava trees (*Psidium guajava* L.) arranged in a circle inside the cage to provide fly habitat. On the first day of testing for each species, we assigned protein-fed flies to two cages and protein-starved flies to the other two cages. On the second day of testing the physiological states were reversed to control for positional effects. Approximately 500 flies were released into each cage at 0900 and 1300 h each day in order to provide a constant supply of responding flies. Before each replicate we misted the foliage of the guava canopy with water to ensure that the flies were not responding to the baits in order to obtain water. Each day we tested flies from 0930 to 1600 h. At the end of each day of testing we flushed the flies from the trees and cages with water from a garden hose.

An Eppendorph (Brinkmann Instruments, Inc., Westbury, NY) repeater pipette was used to apply thirty 10 μl droplets of each treatment to the surface of two leaf strips (2 x 5 cm) of coffee (*Coffea arabica* L.) 5 minutes before the beginning of each test. Leaves had been cut into two strips so that they easily fitted into glass Petri dishes (9 cm diam. x 1.5 cm tall) with screened lids and wire hangers. We attached 22 cm lengths of wire in 5 positions around the perimeter of the tree canopy, which were used to attach to the Petri dish hangers. Treatments were randomly hung in one of the positions at the start of each replicate.

After flies were released inside each cage an observer entered to record the number of male and female flies arriving at each station. Arriving flies showed arrested movement and could be removed from the stations with an aspirator. Every 5 minutes the stations were rotated clock-wise one position, until each treatment had occupied all of the five positions. One replicate test lasted for a total time of 25 minutes.

During each trial we tested four different protein baits and a water control. None of the original bait formulations contained toxicants. The baits were: 1) Mazoferm® E802 (Corn Products, Argo II, USA), 2) Nu-Lure® Insect Bait (Miller Chemical and Fertilizer, Hanover PA, USA), 3) Provesta® 621 autolyzed yeast extract (Integrated Ingredients, Bartlesville, OK, USA), and 4) GF-120 Fruit Fly Bait (Dow-Agro Sciences, Indianapolis, IN). All baits were diluted with water to have an equal viscosity (a specific gravity of 1.192 @ 15.5 °C).

Field cage tests of the attractiveness of the four baits and a water control to the two different *Bactrocera* species were conducted. We observed fly response to the five treatments over a period of 3 days for each fruit fly species. Four replicate cages were used for

the trials. During the testing of each species two cages were randomly designated to contain protein-fed flies and two cages were designated to contain protein-deprived flies. Sixteen replicate trials were conducted for both protein-fed and protein-deprived flies of both species.

Attraction Experiment 2. Residual attractiveness of the four protein baits to the two species of fruit flies was tested on March 30, 2000. Protein-deprived flies were used, since this was the physiological state most likely to respond. Droplets were aged on coffee leaves for a period of 4 days before being used in our cage assay. The aged baits were compared to fresh Provesta and fresh water drops. Baits were aged for 4 d which we selected to be a midpoint time interval in a weekly bait spray program. The starting concentration of all baits was a specific gravity of 1.192 (at 25°C). All baits were aged indoors in a building maintained at $21 \pm 4^\circ\text{C}$, 50–60 % relative humidity, and a 12:12 (L:D) h photoperiod. We conducted 16 replicate tests with *B. dorsalis* and *B. cucurbitae*.

Attraction Experiment 3. Attraction to GF-120 and Provesta baits with malathion or spinosad was compared for both protein fed and protein deprived *B. dorsalis* and *B. cucurbitae* on August 2 and 3, 2000. Malathion treatments contained 20% (AI) malathion and 80% protein bait. Spinosad treatments contained protein baits with 0.01% (AI) spinosad added. Eight replicate trials were conducted for each species in each physiological state. In order to avoid inhaling pesticide fumes, observers removed the flies from the stations by carefully unhooking the dishes and carrying them out of the tent, where flies were brushed aside.

Feeding Experiment 1. Feeding by *B. dorsalis* and *B. cucurbitae* on protein bait droplets was assessed between March 20 and 24, 2000. Protein (Mazoferm, Nu-Lure, Provesta, and GF-120) baits were the same as those used in attraction experiment 1. We cut washed coffee leaves into 2.5 cm² squares and placed them horizontally on small overturned 30 ml plastic beakers (4.5 cm high). These beakers were then placed on a laboratory table under florescent lighting (two 120 cm 40-watt bulbs). One 10 FI drop of a test substance was placed on each leaf square using an Eppendorph repeater pipette. During tests temperature ranged from $22 \pm 4^\circ\text{C}$ and 70–80% relative humidity.

A clear plastic beaker was used to transfer flies from a holding cage after which they were gently nudged from the cup onto the leaf square with the end of a wooden probe. We observed each fly on a leaf square for a maximum time of 600 s. In order to be sure that flies were not agitated from being transferred a minimum staying time of 10 s was established. The trial ended when a fly reached the maximum time limit or flew or crawled off the leaf surface. The duration of feeding bouts on each treatment was recorded and used to calculate a total feeding time for each fly. Each fly was only observed once. One replicate test consisted of one individual fly being tested on each treatment over a period of approximately 30 min. Feeding was timed using a stopwatch and observers recorded data by hand.

Feeding Experiment 2. Tests were conducted between August 29 and September 2, 2000. For each species we conducted trials to examine propensity to feed on protein baits with and without pesticides. We used drops of GF-120 and Provesta to which the pesticides spinosad and malathion had been added, similar to attraction experiment 3. Once again we used water drops as a control. We conducted 10 replicate trials on female flies of both species. Tests containing pesticides were conducted on a bench outside the laboratory where temperature and relative humidity averaged $24 \pm 2^\circ\text{C}$ and $70 \pm 10\%$, respectively.

Flies were handled as described in the first feeding experiment. After observing and recording the feeding behavior of each female, we placed it in a small plastic cup (6 cm high) with a screen mesh top. Each cup contained a 4 cm length of moistened cotton dental wick. A sugar cube was placed on each mesh top. Mortality of 10 flies for each treatment was checked and recorded after 48 h.

Statistical Analysis. For attraction and feeding data, a replicate was considered com-

Table 1. ANOVA of protein-fed and protein-deprived male and female fruit flies, arriving at protein drops.

Source of variation	<i>B. dorsalis</i>			<i>B. cucurbitae</i>		
	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>
Odor	20.06	4	<.0001	21.32	4	<.0001
State	34.75	1	<.0001	0.00	1	0.95
Odor x state	2.29	4	0.06	1.89	4	0.11
Sex	22.42	1	<.0001	12.43	1	<0.001
Odor x sex	1.70	4	0.15	0.14	4	0.97
State x sex	0.04	1	0.84	2.67	1	0.10
Odor x state x sex	0.04	4	1.00	0.19	4	0.95
Error		300				

plete when individual insects had been tested with each treatment during approximately 1-h. For each experiment, data were subjected to an analysis of variance and means were compared using the least significant difference test with $P = 0.05$ (Proc GLM, LSD Test, SAS, 1999).

Results

Attraction Experiment 1. For *B. dorsalis*, effects of odor source, state and sex were highly significant ($P < 0.0001$) (Table 1). None of the interaction terms (odor x state, lure x sex, state x sex, and lure x state x sex) were found to be significant ($P > 0.05$). Protein-fed *B. dorsalis* responded significantly less ($P < 0.0001$) than protein-deprived flies to baits, and female flies were significantly ($P < 0.0001$) more attracted to baits than male flies. Female protein-fed and protein starved *B. dorsalis* flies were significantly ($P = 0.05$) more attracted to Provesta, GF-120, and Mazoferm than to water (Fig. 1). Attraction to Nu-Lure was not significantly ($P > 0.05$) different from water. Male protein fed *B. dorsalis* showed a level of response to all four protein baits equal to that of water. Male protein-starved *B. dorsalis* showed highest attraction to Provesta, GF-120, and Mazoferm compared to water.

In tests with *B. cucurbitae*, effects of odor source and sex were highly significant ($P < 0.001$), while the effect of physiological state was not (Table 1). None of the interaction terms (odor x state, lure x sex, state x sex, and lure x state x sex) were significant. Male *B. cucurbitae* flies were more attracted to protein baits than female *B. cucurbitae* flies. And they responded most strongly to GF-120, Provesta and Mazoferm (Fig. 2). The male response to GF-120 was significantly ($P \leq 0.05$) greater than to Nu-Lure or water. Female *B. cucurbitae* flies were significantly ($P \leq 0.05$) more attracted to GF-120 and Provesta than to Mazoferm, Nu-Lure, or water.

Attraction Experiment 2. In residual tests with *B. dorsalis* the effect of odor source was highly significant ($F = 51.39$; $df = 4, 150$; $P < 0.001$), while the effect of sex was not (Table 2). Males responded most strongly to fresh Provesta, but did not discriminate between water and aged baits. Female *B. dorsalis* responded most strongly to fresh Provesta, whereas they responded equally to 4-day-old Provesta and water.

The effect of odor source was highly significant ($F = 27.86$; $df = 9, 110$; $P < 0.001$) in residual tests with *B. cucurbitae*, as was the effect of sex ($F = 8.33$; $df = 9, 110$; $P = 0.0047$).

Table 2. Number of fruit flies arriving at bait stations.^a

Species	Sex	Treatment	mean $\pm$ SE	
<i>B. dorsalis</i>	Male	Fresh Provesta	15.38 $\pm$ 1.98	A
		Water	7.06 $\pm$ 0.97	B
		4-day old Provesta	4.06 $\pm$ 0.82	BC
		4-day old Nu-Lure	3.19 $\pm$ 0.77	C
		4-day old GF-120	3.06 $\pm$ 0.70	C
	Female	Fresh Provesta	22.56 $\pm$ 2.81	A
		Water	5.31 $\pm$ 0.73	B
		4-day old Provesta	5.12 $\pm$ 1.00	B
		4-day old GF-120	4.00 $\pm$ 0.70	B
		4-day old Nu-Lure	3.44 $\pm$ 0.70	B
<i>B. cucurbitae</i>	Male	Fresh Provesta	26.50 $\pm$ 4.75	A
		Water	10.67 $\pm$ 2.27	B
		4-day old Provesta	6.00 $\pm$ 0.90	B
		4-day old GF-120	6.08 $\pm$ 1.25	B
		4-day old Nu-Lure	5.33 $\pm$ 1.19	B
	Female	Fresh Provesta	18.92 $\pm$ 2.91	A
		Water	5.67 $\pm$ 1.18	B
		4-day old Provesta	3.75 $\pm$ 1.02	B
		4-day old Nu-Lure	3.91 $\pm$ 0.84	B
		4-day old GF-120	3.25 $\pm$ 0.77	B

^a Values in the column by each species, sex, and treatment followed by the same letter are not significantly different at the $P = 0.05$ level (Proc GLM, LSD Test, SAS, 1999).

Interestingly, male response was higher than female response. Males responded over four times stronger to fresh Provesta than to aged baits. Female *B. cucurbitae* response was over four and a half times stronger to fresh Provesta than to the other protein treatments.

Attraction Experiment 3. In studies with *B. dorsalis* the effects of odor source was highly significant ($P < 0.0001$) (Table 3). The effects of sex, physiological state and all interactions were not significant ($P > 0.05$). GF-120 and Provesta with spinosad were significantly ($P \leq 0.05$) more attractive to *B. dorsalis* flies than GF-120 and Provesta with malathion, or water (Table 4).

In studies of *B. cucurbitae*, the effect of odor source and physiological state was highly significant ($P < 0.0001$), while the effect of sex was not. The interaction terms of odor source $\times$ sex, physiological state $\times$ sex, and odor source $\times$ physiological state $\times$ sex were not significant ($P > 0.05$). The interaction of odor source $\times$ physiological state was significant ($P = 0.03$). Protein-deprived flies responded in significantly greater numbers than protein-fed flies. Protein-deprived male and female *B. cucurbitae* arrived in significantly ($P \leq 0.05$) greater numbers to GF-120 and Provesta with spinosad than they did to GF-120 and Provesta with malathion, or to water. Protein-fed male and female *B. cucurbitae* showed significantly ($P \leq 0.05$) more attraction to GF-120 than they did to all other baits, which elicited responses equal to or less than water.

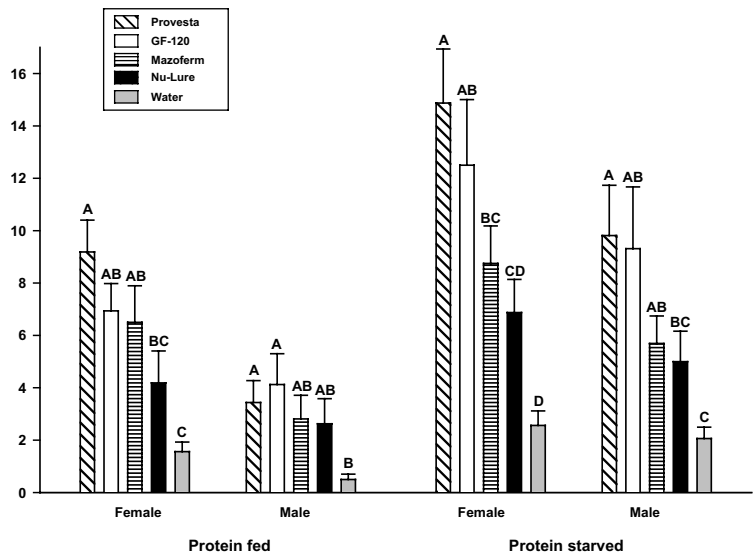


Figure 1. Mean ($\pm$ sem) numbers of male and female protein-fed and protein-starved *B. dorsalis* arriving at bait stations containing protein droplets. Columns headed by the same letter for each sex and state are not significantly different at the $P = 0.05$ level (Proc GLM, LSD Test, SAS, 1999).

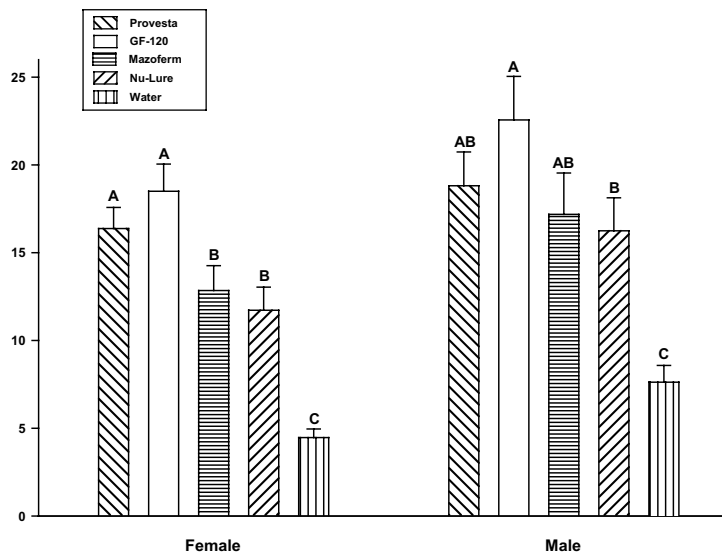


Figure 2. Mean ($\pm$ sem) numbers of male and female *B. cucurbitae* arriving at bait stations containing protein droplets. Responses of protein-fed and protein-deprived flies were pooled. Columns headed by the same letter for each sex are not significantly different at the $P = 0.05$ level (Proc GLM, LSD Test, SAS, 1999).

Table 3. ANOVA of protein-fed and protein-deprived male and female fruit flies, arriving at protein drops with spinosad and malathion.T

Source of variation	<i>B. dorsalis</i>			<i>B. cucurbitae</i>		
	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>
Odor	17.67	4	<.0001	10.56	4	<.0001
State	1.95	1	0.16	20.81	1	<.0001
Odor x state	1.60	4	0.18	2.73	4	0.03
Sex	0.07	1	0.79	0.78	1	0.38
Odor x sex	0.05	4	1.00	0.06	4	0.99
State x sex	3.25	1	0.07	0.70	1	0.40
Odor x state x sex	0.86	4	0.49	0.41	4	0.80
Error		140				

Table 4. Number of fruit flies arriving at bait stations.^a

Species	State	Treatment	mean ± SE	
<i>B. dorsalis</i>	Combined	GF-120 + spinosad	15.66 ± 1.97	A
		Provesta + spinosad	14.19 ± 2.85	A
		GF-120 + malathion	2.88 ± 0.41	B
		Provesta + malathion	3.12 ± 0.62	B
		Water	2.31 ± 0.30	B
<i>B. cucurbitae</i>	Protein-deprived	GF-120 + spinosad	15.25 ± 3.25	A
		Provesta + spinosad	11.63 ± 2.82	A
		GF-120 + malathion	2.81 ± 0.83	B
		Provesta + malathion	2.75 ± 0.87	B
		Water	4.69 ± 1.19	B
	Protein-fed	GF-120 + spinosad	5.43 ± 0.10	A
		Provesta + spinosad	3.56 ± 0.65	B
		GF-120 + malathion	0.25 ± 0.11	BC
		Provesta + malathion	1.31 ± 0.31	CD
		Water	2.69 ± 0.71	D

^a Values in the column by each species state and treatment followed by the same letter are not significantly different at the *P* = 0.05 level (Proc GLM, LSD Test, SAS, 1999).

Feeding Experiment 1. *Bactrocera dorsalis* females fed significantly more (*F* = 9.10; *df* = 1, 110; *P* < 0.001) than *B. dorsalis* males (Table 5). The effects of bait (*F* = 13.52; *df* = 4, 110, *P* = < .001) and bait x sex (*F* = 3.27; *df* = 4, 110; *P* = 0.014) were both significant. Female flies fed significantly (*P* ≤ 0.05) longer on Mazoferm than on any of the other baits. Feeding durations on Provesta and GF-120 were not significantly (*P* > 0.05) different from the water control. *Bactrocera dorsalis* males also fed longest on Mazoferm, but not significantly (*P* > 0.05) more than on Nu-Lure or Provesta. Males did not feed significantly (*P* > 0.05) more on GF-120 than they did on water.

Table 5. Feeding times of *B. dorsalis* and *B. cucurbitae* on protein baits.^a

Species	Sex	Treatment	Seconds (mean $\pm$ SE)	
<i>B. dorsalis</i>	Male	Mazoferm	165.08 $\pm$ 63.16	A
		Nu-Lure	91.25 $\pm$ 34.49	AB
		Provesta	87.17 $\pm$ 47.40	AB
		GF-120	51.91 $\pm$ 22.50	B
		Water	4.33 $\pm$ 1.36	B
	Female	Mazoferm	422.75 $\pm$ 61.15	A
		Nu-Lure	205.17 $\pm$ 64.70	B
		Provesta	128.17 $\pm$ 47.01	BC
		GF-120	45.67 $\pm$ 14.44	C
		Water	6.00 $\pm$ 2.45	C
<i>B. cucurbitae</i>	Male	Mazoferm	121.10 $\pm$ 44.93	A
		Nu-Lure	57.20 $\pm$ 9.91	B
		Provesta	41.00 $\pm$ 15.33	B
		GF-120	48.70 $\pm$ 9.79	B
		Water	8.20 $\pm$ 3.34	B
	Female	Mazoferm	214.92 $\pm$ 53.52	A
		Provesta	138.42 $\pm$ 41.40	AB
		Nu-Lure	99.00 $\pm$ 26.98	BC
		GF-120	80.92 $\pm$ 27.75	BC
		Water	8.67 $\pm$ 4.60	C

^a Values in the column by each species, sex, and treatment followed by the same letter are not significantly different at the $P = 0.05$ level (Proc GLM, LSD Test, SAS, 1999).

Female *B. cucurbitae* fed significantly more ($F = 7.51$, $df = 4$, 109; $P = 0.0073$) than males. The effect of bait was highly significant ($F = 7.47$, $df = 4$, 109; $P < 0.0001$), while the bait $\times$ sex interaction was not. Female *B. cucurbitae* flies fed significantly longer on Mazoferm than on Nu-Lure or GF-120, which did not stimulate more feeding than water. Male *B. cucurbitae* fed significantly longer ($P \leq 0.05$) on Mazoferm than on Nu-Lure, GF-120, Provesta or the water control.

Feeding Experiment 2. For *B. dorsalis* the effect of odor source was highly significant ($F = 4.99$; $df = 4$, 50, $P = 0.0018$)(Table 6). *Bactrocera dorsalis* flies fed significantly ($P \leq 0.05$) longer on GF-120 with spinosad than on other treatments, which all elicited feeding responses similar to water. The effect of odor source was also highly significant for *B. cucurbitae* ($F = 8.39$; $df = 4$, 45, $P < 0.0001$). *Bactrocera cucurbitae* flies fed significantly ($P \leq 0.05$) longer on GF-120 with spinosad than on all other treatments (Table 6).

Discussion

Earlier behavioral studies of *C. capitata* had indicated the importance of physiological state in feeding responses to bait droplets and the unattractiveness of aged protein baits, (Prokopy et al. 1992; Vargas et al. 2001). In subsequent field studies with *B. cucurbitae*,

Table 6. Feeding times of *B. dorsalis* and *B. cucurbitae* on protein baits.^{a,b}

Species	Treatment	Seconds (mean $\pm$ SE)	
<i>B. dorsalis</i>	GF-120 + spinosad	89.18 $\pm$ 31.85	A
	Provesta + spinosad	40.09 $\pm$ 17.33	B
	GF-120 + malathion	0.55 $\pm$ 0.28	B
	Provesta + malathion	4.63 $\pm$ 3.18	B
	Water	13.18 $\pm$ 5.15	B
<i>B. cucurbitae</i>	GF-120 + spinosad	140.10 $\pm$ 42.39	A
	Provesta + spinosad	45.70 $\pm$ 11.50	B
	GF-120 + malathion	6.00 $\pm$ 4.53	B
	Provesta + malathion	9.70 $\pm$ 5.12	B
	Water	5.70 $\pm$ 1.87	B

^aValues in the column by each species and treatment followed by the same letter are not significantly different at the $P = 0.05$ level (Proc GLM, LSD Test, SAS, 1999).

^b*B. dorsalis* and *B. cucurbitae* mortalities for flies feeding on GF-120 (+spinosad), Provesta (+spinosad), GF-120 (+malathion), Provesta (+malathion), and water were 90 and 91%, 89 and 82%, 100 and 100%, 100 and 100%, and 0 and 0%, respectively.

GF-120 Fruit Fly Bait sprayed on a border area proved very effective in preventing protein-deprived females from alighting on cucumbers (Prokopy et al. 2003; 2004). Also, bait spray was highly attractive to protein-deprived females within 1 h of application to sorghum (*Sorghum* sp.), but lost about half of its attractiveness within 5 h, and all of it within 24 h under dry greenhouse conditions. In the present study, physiological state of *B. dorsalis* flies influenced attraction to bait. Only fresh baits were attractive to *B. dorsalis* and *B. cucurbitae* flies.

Vargas et al (2003) suggested that type of protein influenced attraction of flies to the baits. Provesta outperformed the standard Nu-Lure in attractiveness to *C. capitata* flies. Field studies in Hawaii further suggested that Provesta and Mazoferm could be used in bait sprays for suppression of *B. dorsalis* in guava orchards (McQuate et al., 1999) and *C. capitata* in coffee fields (Peck & McQuate, 2000), respectively. The present study also suggests that protein type was important for attraction of both *B. dorsalis* and *B. cucurbitae*. GF-120 was in the top group of proteins screened and generally rated higher than Nu-Lure. Aged baits, when compared to fresh baits, were unattractive to *B. dorsalis* and *B. cucurbitae*. Since attractiveness of bait droplets is short-lived, baits should be applied at short intervals or other ingredients added to baits to extend the period of attractiveness. Weekly applications of baits would likely be the shortest spray interval that is economically feasible for farmers. Bait degradation needs to be investigated further to determine if loss of attraction is due to the protein or other additives such as ammonium acetate.

The current study provides data on attraction and feeding by the two most economically damaging species of fruit flies present in Hawaii and behavioral responses will be compared with wild lines in future studies. Although laboratory strains were used in these evaluations, we feel that the observed responses will be generally representative of wild populations. To substantiate this claim, future studies will use similar methodologies to evaluate the behavioral responses of wild *B. cucurbitae* and *B. dorsalis* to protein baits. Our data

clearly indicate that protein baits need to be examined in the context of both attraction and feeding response. For example, Provesta rated very high with respect to attraction, while Mazoferm rated very high with respect to feeding. GF-120 rated high with respect to attraction, but low with respect to feeding. On the basis of both attraction and feeding, only Mazoferm rated higher than the standard Nu-Lure. Our feeding and attraction data indicate that spinosad could potentially replace malathion in protein bait sprays for *B. dorsalis* and *B. cucurbitae*. However, more field testing is required for validation.

In summary our results suggest that spinosad is a promising substitute for organophosphate insecticides in protein bait sprays for control of *B. dorsalis* and *B. cucurbitae*. Present research in Hawaii is focusing on integration of techniques and a reduction in the use of organophosphate insecticides, such as reduced risk bait sprays combined with male annihilation, for area-wide Integrated Pest Management (IPM) of fruit flies (i.e., *B. dorsalis* and *B. cucurbitae*). The development of IPM approaches that include the use of both protein bait sprays and male annihilation dispensers without organophosphates (Vargas et al. 2000) would have important applications to suppression of fruit flies not only in Hawaii but also throughout the south and western Pacific, Australia, and tropical Asia where *Bactrocera* are serious economic pests. Finally, the development of environmentally-friendly area-wide IPM procedures would have important applications to eradication of accidental introductions of fruit flies into the U.S. mainland. Spinosad bait sprays may be a viable alternative to malathion that could be integrated with sterile fly releases.

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Footnotes

¹ This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation by the USDA for its use.

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